L. L. NARAYANA*: Contributions to the embryology of Balsaminaceae (2)**

ナラヤナ，エル・エル： ツリフネソウ科の発生学的研究 (2)

Introduction After Schnarf's review (1931) on the embryological features of Balsaminaceae, a few more contributions have appeared on the embryology of this family (Dahlgren, 1934; Steffen, 1946; Venkateswarlu & Lakshminarayana, 1957; Narayana & Sayeeduddin, 1959; Narayana, 1963). The present study which is a further extension of studies on the embryology of this family relates to a comparative account of the development of male and female gametophytes in *Impatiens maculata* Wight, *I. oliveri* C. H. Wright ex W. Wats., and *I. parviflora* DC., and endosperm and embryo in *I. levengi* Gamble ex Hook. f., *I. arguta* Hook. f. & Thoms., *I. leschenaultii* Wall., and *I. radiata* Hook. f.

Material and method All the material was fixed in formalin acetic alcohol. Customary methods were followed for processing the material. Endosperm haustoria were studied with the help of suitable dissections.

Microsporogenesis and male gametophyte The structure and development of the anther in six species of *Impatiens* have been already described (Narayana, 1963). So, only the salient features are given for the plants mentioned in the present study. The anther wall is made up of the epidermis followed by four to five wall layers (fig. 1). The innermost of these, which functions as the tapetum, surrounds the sporogenous tissue (fig. 1). Some of the sporogenous cells get sterilized and contribute towards the formation of trabeculae (fig. 1). The endothecium develops from the hypodermal wall layer and the middle layers are crushed during development. Cytokinesis takes place by furrowing and both isobilateral and tetrahedral arrangement of pollen tetrads have been observed (figs. 2-5). Figure 6 shows a young pollen grain with a lenticular generative cell. The pollen grains are 4-colpate and are 2-celled at the time of shedding (figs. 7,8). 'Onci' occur in *I. maculata*, *I. parviflora* and *I. oliveri* (fiig. 7). As the pollen grains mature, they flatten and the generative cell appears elongated with tapering ends (fig. 8).

Ovule The ovule is tenuinucellate, bitegminous and anatropous (figs. 9-14).

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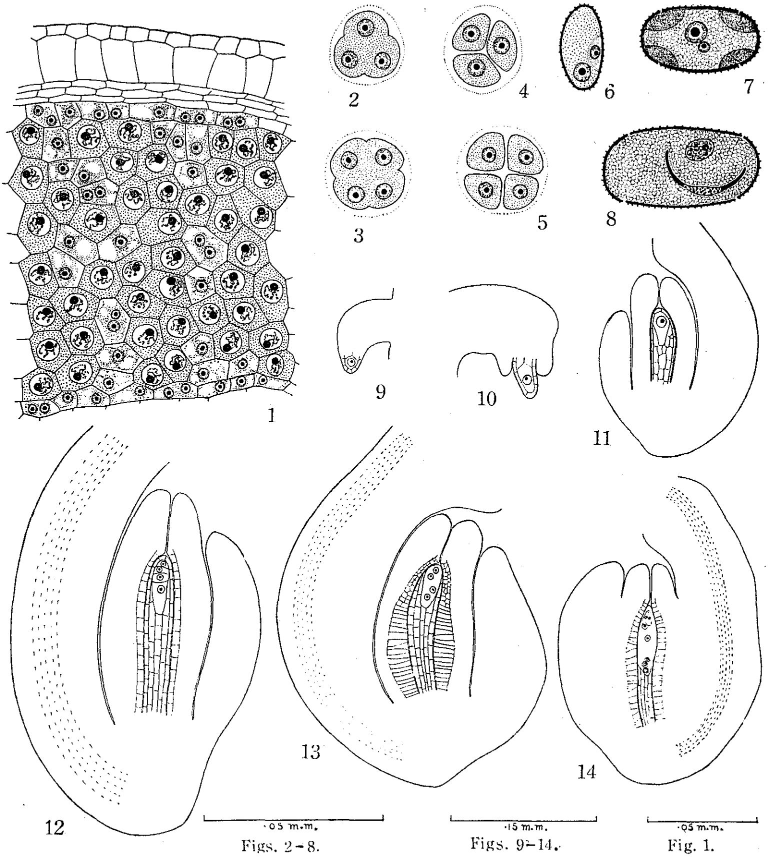
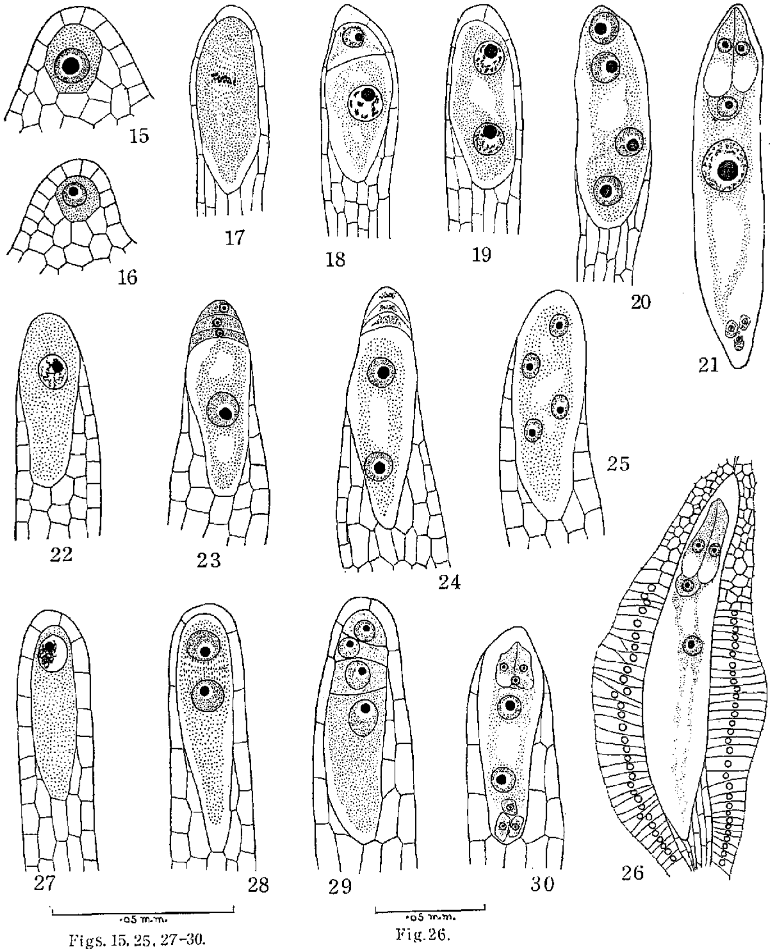


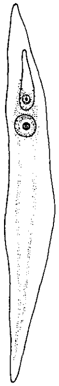
Figs. 1-14. 1, 9-11, 13. *I. maculata.* 2-5, 14. *I. oliveri.* 6-8, 12. *I. parviflora.* 1. l. s. anther lobe showing wall layers, tapetum and sporogenous tissue. Note sterilized sporogenous cells. 2, 3. p. m. c. showing cytokinesis. 4, 5. tetrahedral and isobilateral pollen tetrads. 6. young pollen grain showing the lenticular generative cell. 7. pollen grain showing 'onci'. 8. mature pollen grain. 9-11. stages in the development of the ovule. 12-14. mature ovules.

The nucellus though scanty is long, narrow and differentiates at the tip of the prominent funiculus (fig. 9). During development, the ovule undergoes a curvature, and becomes anatropous with the raphe on the dorsal side (figs. 10–14). The integuments in *I. oliveri* are fused throughout except for a short distance at the apex where they are free (fig. 14). *I. maculata* and *I. parviflora* have free integuments (figs. 12, 13). The micropyle is formed only by the inner integument (figs. 12–14). An endothelium is differentiated from the innermost layer of the inner integument (figs. 12–14). The nucellus is completely crushed by the developing embryo sac.

Megasporogenesis and female gametophyte The hypodermal archesporium in the ovule directly functions as the megaspore mother cell (figs. 15–17,



Figs. 15-30. 15, 17-21. *I. oliveri.* 16, 22-26. *I. maculata.* 27-30. *I. parviflora.* 15, 16. primary archesporium. 22, 27. megaspore mother cell stage. 17, 28. megaspore mother cell in division. 18. dyad stage. 23, 29. linear megaspore tetrad. 19, 24. 2-nucleate stage of embryo sac. Note degenerating megaspores in 24. 20, 25. 4-nucreate stages of embryo sac. 21, 30. organized embryo sacs. 26. mature embryo sac.



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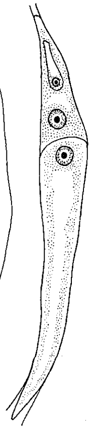

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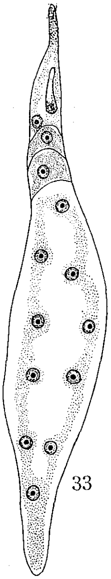

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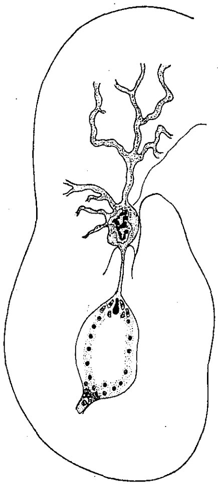

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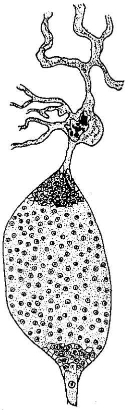

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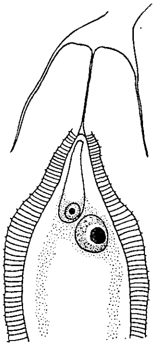

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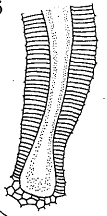

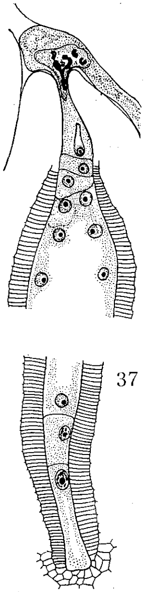

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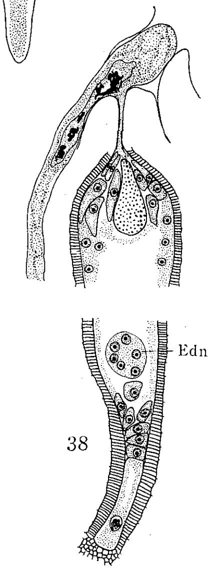

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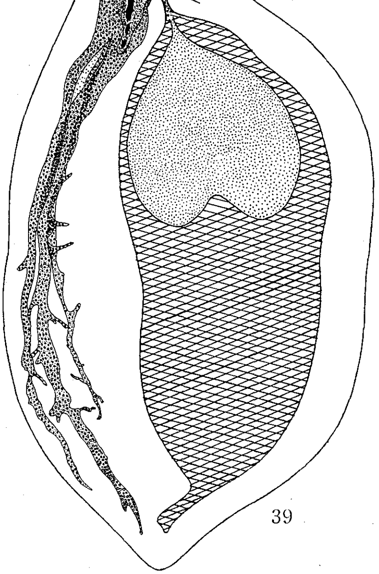

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·5 m.m. Figs. 34,35,39.

·1 m.m. Figs. 31–33, 36, 37.

·15 m.m. Fig. 38.

22, 27). *I. oliveri* it divides to form dyads, of which the lower is larger and functional (figs. 17, 18); the nucleus in this cell after undergoing three successive free nuclear divisions gives rise to an 8-nucleate embryo sac (figs. 19-21). Thus, the development of the embryo sac follows the *Allium* type (Maheshwari, 1950). In *I. maculata* and *I. parviflora* the megaspore mother cell as a result of meiosis gives rise to a linear tetrad of megaspores (figs. 23, 28, 29), of which the lowermost functions and gives rise to an 8-nucleate embryo sac of the *Polygonum* type (figs. 23-26, 29, 30). The egg apparatus consists of two synergids and an egg. The antipodals degenerate early and the polars fuse before fertilization (fig. 26). The endothelium surrounds the mature embryo sac (fig. 26).

Endosperm The primary endosperm nucleus lies in the upper part of the elongated embryo sac (figs. 31, 36). It divides before the fertilized egg by a transverse wall (figs. 32, 41). The embryo sac is thus divided into a small upper micropylar and a large lower chalazal chamber (figs. 32, 41). Transverse devisions in the micropylar chamber result in a superposed row of cells (figs. 33, 37, 42, 45, 46). At this stage the embryo sac shows a micropylar chamber enclosing the zygote, two middle chambers and the large chalazal chamber (figs. 33, 42, 45, 46). For some time only free nuclear divisions occur in the chalazal chamber (figs. 33, 42, 46). Later cytokinesis sets in at its lower end and a row of two or four cells is formed (figs. 37, 38, 43). The lowermost cell of this row is large and encloses dense cytoplasm and a hypertrophied nucleus; it functions as the chalazal endosperm haustorium (figs. 34, 35, 37, 38, 43, 47).

The further behaviour of the micropylar chamber is interesting. It develops into a giant micropylar haustorium before the zygote undergoes division (figs. 11, 37). During development, the micropylar chamber along with its

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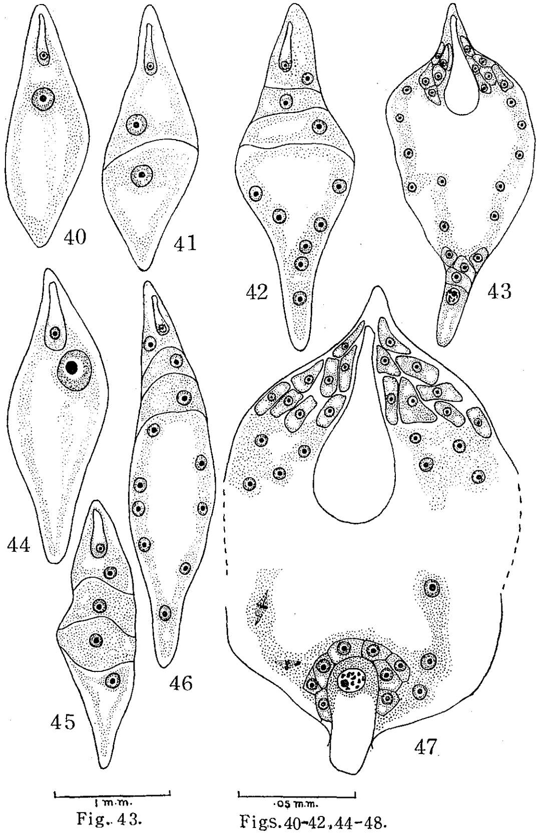
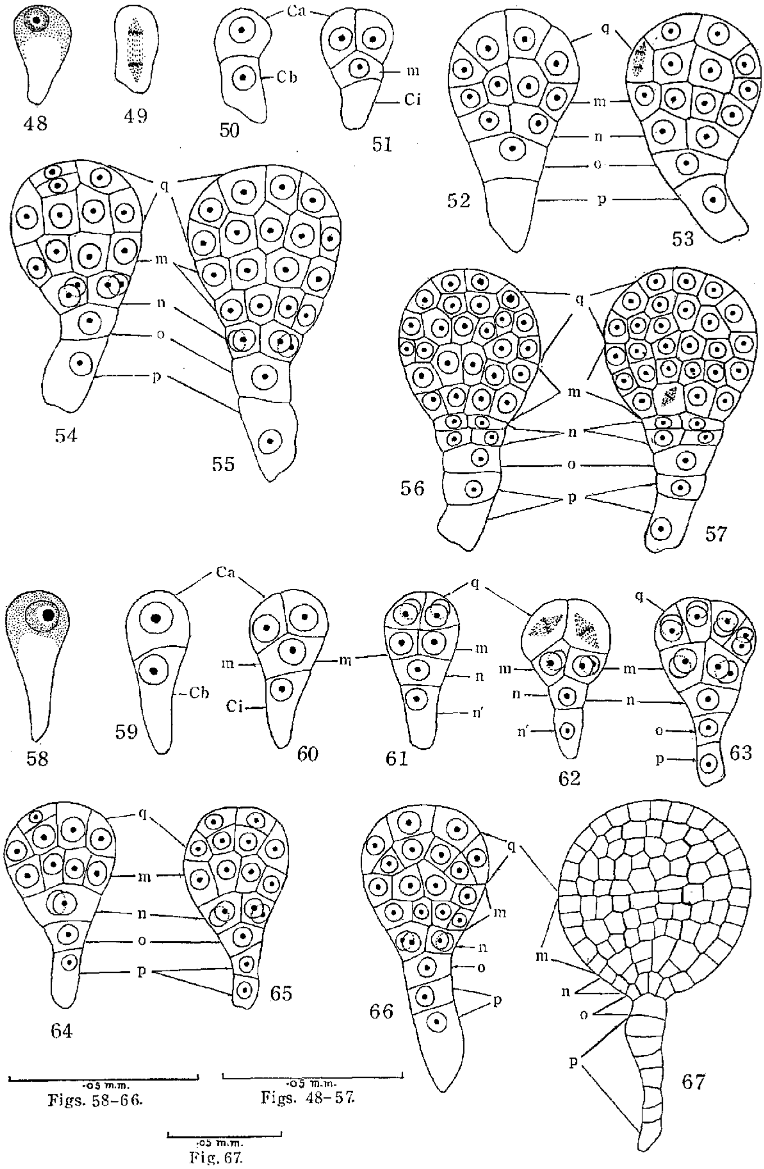
Figs. 31-39. 31-35. *I. leshenaultii.* 36-39. *I. radiata.* 31, 36. embryo sac showing zygote and endosperm primordium. 32. formation of micropylar and chalazal chambers. Note smaller micropylar chamber. 33. advanced stage, showing the large chalazal chamber and 3 cells formed from the micropylar chamber. Note growing upper chamber and free endosperm nuclei in the chalazal chamber. 34. l. s. ovule showing the chalazal and micropylar haustoria, micropylar and chalazal cellular endosperm and the middle free nuclear endosperm. (reconstructed). Micropylar haustorium ramifying in the placenta. 35. dissection of embryo sac showing the aggressive micropylar haustorium and the chalazal haustorium. The micropylar and chalazal cellular endosperm and the middle free nuclear endosperm also seen. 37. l. s. ovule showing micropylar and chalazal haustorium and middle free nuclear endosperm. Note zygote is undivided. 38. same as above at an advanced stage. Note micropylar and chalazal endosperm tissue and endosperm nodule above the chalazal endosperm tissue (Edn). 39. seed cleared in chloral hydrate showing the much branched endosperm haustorium reaching the chalazal end.



Figs. 40-47. 40-44. *I. arguta.* 45-47. *I. levengi.* 40. embryo sac showing zygote and primary endosperm nucleus. 41. formation of micropylar and chalazal chambers. 42, 45 46. advanced stage showing the large chalazal chamber and three cells formed by divisions of micropylar chamber. Note undivided zygote and free endosperm nuclei in the chalazal chamber in 42 and 46. 43, 47. embryo sac showing advanced embryo. Note absence of micropylar haustorium and presence of chalazal endosperm haustorium. Micropylar and chalazal endosperm tissue and free endosperm nuclei in the middle chamber also seen.

nucleus pushes its way out through the micropyle (fig. 33). It now becomes vesicular, and soon produces branches which ramify in the funicular and placental tissues (fig. 34, 35). The nucleus becomes hypertrophied and irregular in outline and lies in the vesicular part of the haustorium above the micropyle (figs. 34, 35). In *I. radiata,* the haustorium does not penetrate the placentral tissue, but pierces into the fused integuments along the side of the raphe; soon



Figs. 48-67. 48-57. *I. levengi*. 58-67. *I. arguta*. Stages in the embryo development. For abbreviations see text.

its branches extend towards the chalaza and lie close to the funicular vascular bundle (figs. 37-39).

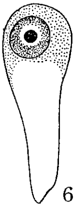
I. levengi and *I. arguta* differ from *I. leschenaunltii* and *I. radiata* in the arrested behaviour of the micropylar haustorium (figs. 43, 47). The development of the chalazal haustorium is as in the above mentioned species (figs. 43, 47).

Whether the micropylar haustorium differentiates or not, an endosperm tissue is organized by the two middle tiers of cells (figs. 34, 35, 37, 38, 43, 47). The endosperm at this stage consists of a giant micropylar haustorium (except in *I. arguta* and *I. levengi*), a cellular endosperm surrounding the embryo, a middle free nuclear endosperm and the chalazal haustorium (figs. 34, 35 37, 38, 43, 47). During these changes the embryo sac becomes widened and the chalazal haustorium appears to push its way into the cellular endosperm formed above it (figs. 35, 47).

Another interesting feature in the endosperm is the occurrence of few endosperm nodules in the free nuclear endosperm. A nodule consists of a few free nuclei surrounded by a common cytoplasmic vesicle (fig. 38).

Embryo The first division of the zygote (figs. 48, 58, 68, 74) is transverse resulting in a apical cell *ca*, and a basal cell *cb* (figs. 49, 50, 59). Cell *ca*, divides vertically and gives rise to two juxtaposed cells, while *cb* divides transversely forming two superposed cells *m* and *ci* (figs. 51, 60, 69, 75). The two cells of *ca* divide vertically resulting in 4 circumaxially arranged cells which constitute the tier *q* (figs. 61, 70, 76, 77). Now *m* divides by a vertical wall and *ci* divides transversely giving rise to two cells *n* & *n'* (figs. 61, 62, 76). Cells of the tier *q* divide by oblique walls to form the octants (figs. 52, 62, 63, 71, 78) and cells of the tier *m* divide vertically forming four circumaxially arranged cells (figs. 62, 63, 70, 78). At about this stage *n* divides vertically into two juxtaposed cells (figs. 52, 64, 71, 78) and *n'* transversely to form two superposed cells *o* and *p* (figs. 52-55, 63-65, 70, 71, 77-79).

Soon, the dermatogen initials are laid down in tiers *q* and *m* as a result of periclinal divisions (figs. 53-55, 64, 65, 72, 79). Further divisions result in the initiation of plerome and periblem in the tiers *q* and *m* (figs. 56, 57, 66, 67, 73, 80). The derivatives of the tier *q* give rise to the stem tip and cotyledons, while the derivatives of *m* give rise to the hypocotyl and plerome initials of the root. The derivatives of *n* contribute towards the formation of the root cortex and the apical part of the root, and the root cap is formed by the derivatives of *o*.



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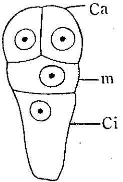

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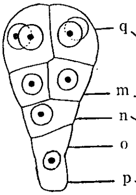

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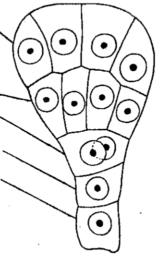

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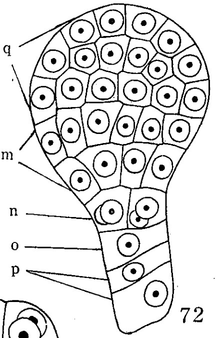

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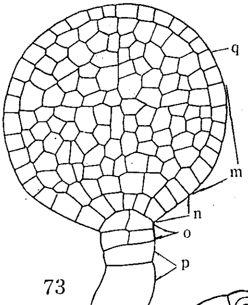

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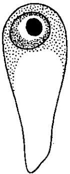

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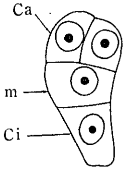

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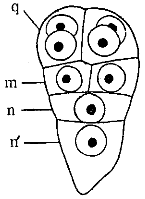

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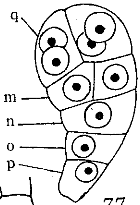

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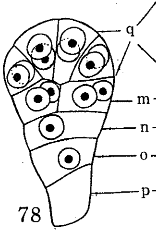

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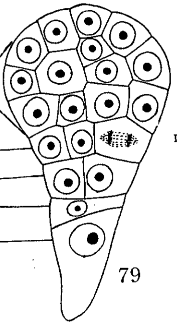

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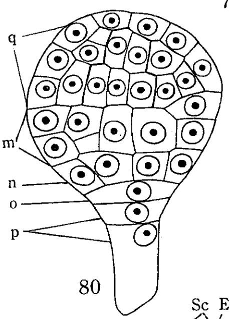

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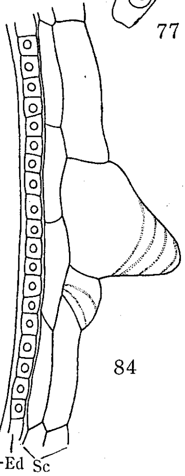

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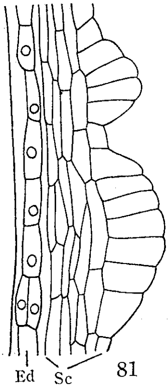

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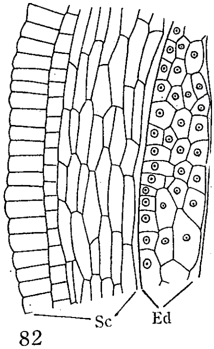

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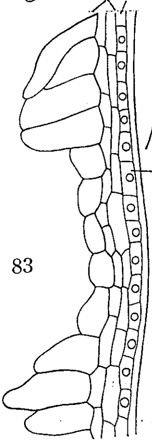

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·05 m.m.
Figs. 68–80.

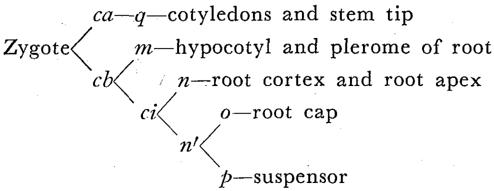
·15 m.m.
Figs. 81–84.

·05 m.m.
Fig. 73.

The suspensor develops from tier *p* and is usually short except in *I. arguta*, where it is filamentous consisting of five to seven cells (fig. 67).

Since both *ca* and *cb* contribute to the formation of the embryo, the embryo development on broad lines conforms to the Asterad type (Johansen, 1950). The derivatives of *m* engender the hypocotyl and plerome of the root, recalling the *Senecio* variation under this type.

The following schematic representation shows the derivatives of the different embryonic tiers.


Zygote
ca—q—cotyledons and stem tip
cb
m—hypocotyl and plerome of root
ci
n—root cortex and root apex
n'
o—root cap
p—suspensor

Fruit and seed The fruit in *Impatiens* is a capsule. The seed coat in the plants studied consists of 2–5 layers of thin-walled cells (figs. 81–84). The epidermal cells become papillose in *I. levengi*, *I. arguta* and *I. radiata* (figs. 81, 83, 84), or radially elongated in *I. leschenaultii* (fig. 82). Some of the papillose cells in *I. levengi* show fibrous thickenings, which stain with crystal violet (fig. 84). The persisting endosperm is one layered in *I. arguta*, *I. levengi* and *I. radiata* (figs. 81, 83, 84) or three to four layered in *I. leschenaultii* (fig. 82). The seeds are thus endospermic. The erect embryo has a short stalk and is centrally placed with their closely appressed cotyledons.

Discussion In the anther the formation of trabeculate partitions which divide the sporogenous tissue into islands, appears to be a characteristic feature of the Balsaminaceae. In *Impatiens* they arise from the sporogenous tissue, in *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957) their origin is from the parietal wall layers of the anther. The generative cell is usually elongated except in *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957) where it is small and round.

Tenuinucellate, bitegminous and anatropous ovules with a dorsal raphe are

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Figs. 68-84. 68-73, 82. *I. leschenaultii*. 74-80, 83. *I. radiata*. 81. *I. arguta*. 84. *I. levengi*. 68-73, 74-80. stages in embryo development. For abbreviations see text. 81-84. seed coat in sectional view. Note papillose epidermal cells of seed coat in 81, 83, 84 and radially elongated cells in 82. Also note single layer of endosperm tissue in 81, 83, 84 and 3-4 layers in 82. (Sc: seed coat. Ed: endosperm).

seen in the various taxa of the family investigated so far (Dahlgren, 1934; Venkateswarlu & Lakshminarayana, 1957; Narayana & Sayeeduddin, 1959; Narayana, 1963). The integuments are free in *I. maculata*, *I. parviflora* (present study) and *I. pallida* (Raitt, 1916); but in others they become united throughout except at the apex (Dahlgren, 1934; Venkateswarlu & Lakshminarayana, 1957; Nayayana & Sayeeduddin, 1959; Narayana, 1963). The solitary report of the occurrence of a parietal cell in *I. pallida* (Raitt, 1916) requires reinvestigation.

Allium as well as *Polygonum* types of embryo sac development occur in the family: the former in *I. oliveri* (present study), *I. roylei* (Dahlgren, 1934), *I. leschenaultii* (Narayana & Sayeeduddin, 1959), *I. arguta* (Narayana, 1963) and *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957); the latter in *I. maculata*, *I. parviflora* (present study), *I. levengi*, *I. tenella*, *I. radiata*, *I. tripetala* and *I. inconspicua* (Narayana, 1963).

In the species so far investigated the micropylar endosperm haustorium is prominent and aggressive; the chalazal haustorium is not so prominent. However, *I. arguta* and *I. levengi* differ from the rest of the species in the arrested development of the micropylar haustorium. The micropylar haustorium in various species of *Impatiens* is uninucleate, but multinucleate in *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957). An interesting feature about the endosperm in Balsaminaceae is the occurrence of endosperm nodules. Such nodules are present in *I. leschenaultii*, *I. arguta*, *I. levengi*, *I. radiata* and have also been reported in other species of this family like *I. roylei* (Dahlgren, 1934) and *Hydrocera* (Venkateswarlu & Lakshminarayana, 1957).

An Asterad type (Johansen 1950) of embryo development has been reported in *I. balfourii* (Souèges, 1945), *I. glanduligera* (Steffen, 1946) and *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957); in *Impatiens* is conforms to the *Senecio* variation (present study), and in *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957) it keys out to the *Erodium* variation under this type.

The fruit is a capsule in *Impatiens*, while in *Hydrocera triflora* it is reported as a drupe (Hooker et. al., 1875, Gamble Venkateswarlu & Lakshminarayana, 1957). Recently, Venkateswarlu & Dutt (1961) have redescribed the fruit in *Hydrocera triflora* as a "capsular berry dehiscing septicidally leaving the seeds attached to the column but separating later". The seeds of various species of *Impatiens* are endospermic while in *Hydrocera triflora* they exalbuminous (Venkateswarlu & Lakshminarayana, 1957; Venkateswarlu & Dutt, 1961).

The seed coat structure in the two genera of the family varies. It is made up of two to three layers thin-walled cells in the taxa studied here, except *I. leschenaultii* where it is 5-6 layered. The cells of the epidermal layer become papillose in *I. arguta*, *I. levengi* and *I. radiata*, while they get radially elongated in *I. leschenaultii*. In *Hydrocera triflora* (Venkateswarlu & Lakshminarayana, 1957) the seed coat shows an outer group of brown thick-walled cells and an inner thick-walled tissue. The erect embryo with thick cotyledons occupies a central position in the seed, and thus the internal morphology of the seed is of the spathulate type (Martin, 1946).

Summary

1) The anther wall consists of an epidermis, endothecium, two middle layers and tapetum. The sterilized sporogenous cells form trabeculae. Tetrahedral and isobilateral pollen tetrads have been observed. Mature pollen is 2-celled at the shedding stage.

2) The ovule is tenuinucellate, bitegminous and anatropous. The integuments are free throughout in *I. maculata* and *I. parviflora*, but are almost fused except for a short distance at the apex in *I. oliveri*.

3) The embryo sac develops according to the *Allium* type in *I. oliveri*, and *Polygonum* type in *I. maculata* and *I. parviflora*.

4) The endosperm is *ab initio* cellular. A giant micropylar endosperm haustorium is organized in *I. leschenaultii* and *I. radiata*, while it is absent in the other two species. However, a chalazal endosperm haustorium is noticed in all the species, though it is not so aggressive as the micropylar one.

5) Embryo development conforms to the *Senecio* variation of the Asterad type.

6) Fruit is a capsule with albuminous seeds which are of the spathulate type. The epidermal cells of the seed coat become papillose or radially elongated, and in *I. levengi* some of them show fibrous thickenings.

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第1報（インド植物学会々誌 **42**: 102–109, 1963）に続き，ツリフネソウ科植物の有性世代発生の比較研究を報告する。材料にはツリフネソウ属の7種を用いた。

1) 葯室内では胞子母細胞群の一部が無性化して隔壁を作り，花粉母細胞群を仕切ってしまう。4 面体と 2 面体の両様の花粉粒が見られる。

2) 胚珠は倒生，2 珠皮，珠心組織は薄い。2 枚の珠皮がほとんど全体にわたり合着する種類と，全く合着しないものとがある。

3) 胚のう発生の様式には *Allium* 型と *Polygonum* 型とが認められる。

4) 内乳は初めから細胞性である。種類によっては大きな吸収組織が珠孔から出て珠柄内に伸びてくるが，着点方向の吸収組織は常に形成される。

5) 胚発生は *Aster* 型の中の *Senecio* 型に属する。

6) 胚が真直で内乳の中央に位置するので，種子は spathula 型である。乳頭状に突出した種皮の細胞の中には膜に肥厚模様を示すものがある。